Journal of Medical Virology 60:468–474 (2000)

# Evaluation of the Antibody Specificities of Human Convalescent-Phase Sera Against the Attachment (G) Protein of Human Respiratory Syncytial Virus: Influence of Strain Variation and Carbohydrate Side Chains

**Concepción Palomo,[1]* Patricia A. Cane,[2] and Jose A. Melero[1]**

[1]*Centro Nacional de Biología Fundamental, Instituto de Salud Carlos III, Madrid, Spain*
[2]*Division of Immunity and Infection, University of Birmingham Medical School, Birmingham, England*

The C-terminal third of the attachment protein (G) of several human respiratory syncytial virus isolates was obtained as either a glycosylated protease-resistant fragment of the purified protein or a nonglycosylated GST fusion protein expressed in bacteria. The reactivity of human convalescent-phase sera with both forms of the protein segment was evaluated in immunoblots. While all serum samples reacted with the mature intact protein of the different isolates, only certain samples reacted with the nonglycosylated C-terminal segment of some viral isolates. The number of human serum samples reacting with the glycosylated C-terminal fragment was even more limited. These results highlight the heterogeneity of the human antibody response against epitopes located in the C-terminal hypervariable region of the G molecule and the influence of carbohydrate side chains for expression of these epitopes. We also have analysed the specificities of human sera by competitive enzyme-linked immunosorbent assay with murine monoclonal antibodies (MAbs). Most human serum samples inhibited virus binding of MAbs that recognised conserved or group-specific epitopes of the G protein, while only a limited fraction of those samples inhibited binding of MAbs that recognised strain-specific epitopes. These results are discussed in terms of the antibody repertoire induced after human respiratory syncytial virus infection and the relevance of escape mechanisms to preexisting antibodies for the evolution of this virus. ***J. Med. Virol. 60:468–474, 2000.*** 

**KEY WORDS:** respiratory syncytial virus; attachment (G) protein; human antibodies; carbohydrate side chains

## INTRODUCTION

Human respiratory syncytial virus (HRSV) is the single most important cause of severe lower respiratory tract infection in babies and young children and also is considered a serious problem in the elderly [reviewed by Collins et al., 1996]. Seroepidemiologic data and studies done with laboratory animals indicate that neutralising antibodies mediate protection against HRSV infection. These antibodies recognise either the attachment protein (G) that interacts with the still unknown cell surface receptor or the other major viral glycoprotein (F) that mediates membrane fusion.

The G molecule is a type II glycoprotein with a single hydrophobic domain between residues 38 and 66 that serves as both membrane anchor and signal sequence. The C-terminal ectodomain has a central region (residues 164 to 176) that is conserved among all human strains, and it is a candidate for the receptor-binding site [Johnson et al., 1987]. Flanking this region, there are two protein segments that have a high level of sequence variation among different virus isolates [Cane et al., 1991; Sullender et al., 1991; García et al., 1994]. The 32-kd G-protein precursor is modified extensively by the addition of N- and O- linked oligosaccharide

Grant sponsor: European Union; Grant number: ERBIC18CT-980374; Grant sponsor: Ministerio de Educación y Cultura; Grant number: PM 96-0025; Grant sponsor: Fondo de Investigaciones Sanitarias; Grant number: 98/1086; Grant sponsor: Wellcome Trust.

*Correspondence to: Concepción Palomo, Unidad de Biología Viral, Centro Nacional de Biología Fundamental, Instituto de Salud Carlos III, Majadahonda 28220, Madrid. Spain.

Accepted 25 August 1999

chains to yield the mature molecule of 80 to 90 kd [Wertz et al., 1989; Collins and Mottet, 1992]. Approximately 15% of the G protein synthesized in infected cells is initiated at a second AUG. This second form, named G soluble (Gs) protein, lacks the cytoplasmic region and part of the transmembrane sequence, and it is secreted into the medium after removal of the N-terminal hydrophobic peptide [Hendricks et al., 1988; Roberts et al., 1994].

HRSV isolates have been classified into two antigenic groups by their reactivity with panels of monoclonal antibodies (MAbs) [Anderson et al., 1985; Mufson et al., 1985]. Three types of epitopes, recognised by murine MAbs, have been identified in the G protein: conserved epitopes, present in all HRSV isolates; group-specific epitopes, shared by all viruses of the same antigenic group; and strain-specific or variable epitopes, present only in certain isolates of the same antigenic group [Martínez et al., 1997].

Early attempts, using synthetic peptides, to locate epitopes recognised by antibodies present in human convalescent-phase sera in the G primary structure identified only peptides from the central conserved region [Norrby et al., 1987]. Moreover, human sera failed to inhibit virus binding of murine MAbs whose epitopes had been mapped to the C-terminal variable region of the G molecule [Palomo et al., 1991]. Since the G protein shows the largest sequence differences between human isolates, failure to find human antibodies specific to G variable regions could be explained by differences in the viral genotype. By matching the sequences of synthetic peptides to those of the infecting virus genotype, three peptides of the C-terminal third of the G protein were identified that reacted with convalescent-phase serum samples from babies. This reactivity was abrogated by the introduction of amino acid changes found in other viral genotypes [Cane, 1997]. Using GST fusion proteins covering the 84-85 C-terminal residues of the G protein, it was also established that the human antibody response to that protein segment is closely related to the infecting genotype [Cane et al., 1996].

Carbohydrate side chains also contribute to the antigenic structure of the G molecule. Thus, certain MAbs do not react with the nonglycosylated G-protein precursor [Palomo et al., 1991], or they depend on cell-type-specific glycosylation for antigen recognition [García-Beato et al., 1996]. In an attempt to evaluate the importance of sugars to the immune response of humans against the HRSV G protein, we analysed the reactivity of human convalescent-phase serum samples with the C-terminal third of the G protein either glycosylated or depleted of sugars. The results obtained indicate that carbohydrates can either mask certain antigenic sites or enhance the reactivity of certain antibodies with the G protein. In addition, enzyme-linked immunosorbent assay (ELISA) competition tests indicate that antibodies with specificities similar to those of murine MAbs can be found in human sera.

## MATERIALS AND METHODS

### Serum Samples

Serum samples from patients admitted with respiratory disease to different hospitals in Madrid between 1991 and 1994 were tested for HRSV infection by a complement-fixation assay. Thirty-five serum samples considered positive for anti-HRSV antibodies (CF titre >64) were included in this study. The ages of the patients ranged from 18 months to 88 years.

### Monoclonal Antibodies

MAbs with the prefix 021/ were raised against the Mon/3/88 strain and the other antibodies against Long virus. Their preparation and characterization have been described [García-Barreno et al., 1988; Martínez et al., 1997].

### Viruses

The origin of the viruses used in this study has been described [García et al., 1994]. Their G-protein genes were sequenced, and their phylogenetic relatedness was analysed using statistical methods [García et al., 1994]. The following viruses, representing different branches of the phylogenetic tree of antigenic group A, were selected for this study: strains Mon/3/88, Mad/2/88, Mad/3/89, Mad/5/92, Long, and Mad/8/92. Strains Mon/3/88, Mad/2/88, Mad/3/89, and Mad/5/92 have two consecutive termination codons after nucleotide 906, thus encoding G proteins of 297 amino acids. Strains Long and Mad/8/92 have mutations of the first termination codon, thus generating G proteins of 298 amino acids. All viruses were grown in Hep-2 cells, as previously described [García-Barreno et al., 1988].

### Cell Extracts

Monolayers of Hep-2 cells infected with virus were scraped off the culture plates at 36–48 hr after infection. The cells were sedimented by centrifugation at low speed. After washing with phosphate-buffered saline (PBS), cell pellets were resuspended in lysis buffer (10 mmol/L Tris HCl at pH 7.6, 140 mmol/L NaCl, 5 mmol/L EDTA, 1% octylglucoside), and the extracts were clarified by centrifugation in a microcentrifuge.

### Purification of G Soluble Protein and Protease Digestion

Shed Gs was purified from culture medium of infected cells. One in 10 culture plates was labeled with [$^3$H]glucosamine, as previously described [García-Barreno et al., 1989], to follow protein purification by radioactive counts. Proteins in the clarified supernatant of infected cells were precipitated with 65% $(NH_4)_2SO_4$. The protein pellet, resuspended in buffer A (20 mmol/LTris HCl at pH 7.5, 500 mmol/L NaCl, 0.2% octylglucoside ), was dialyzed against the same buffer and loaded on an immunoaffinity column. This column was made with MAbs directed against conserved epitopes of the G protein bound to CNBr-activated Sepha-

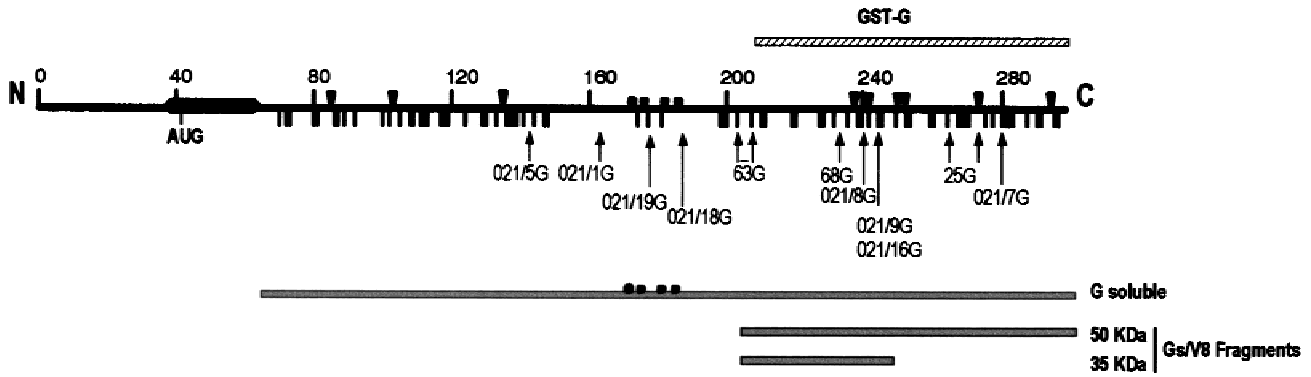


Fig. 1. Scheme of the primary structure of the G protein. The initiation codon of the G soluble protein (AUG), the transmembrane region (thick line), cysteine residues (●), potential sites for N- (▼) and O-glycosylation ( | ) in the Long strain, and the location of murine MAb epitopes (↑) used in this study are indicated. The segments corresponding to the Gs protein and proteolytic fragments derived from it are shown at the bottom. The segment cloned as GST fusion protein is indicated above the protein diagram.

rose [García-Barreno et al., 1989] and equilibrated in buffer A. After washing with the same buffer, the bound material was eluted with 100 mmol/L glycine-HCl at pH 2.5, 200 mmol/L NaCl, and 0.2% octylglucoside. Fractions with the highest level of $^3$H were pooled, neutralized with saturated Tris, and kept at –20°C. Aliquots of Gs, equivalent by immunoblot to the amount of G present in 5 μg of extracts of infected cells, were digested with 1 μg of *Staphylococcus aureus* V8 protease for 1 hr at 37°C in 10 mmol/L Tris-HCl at pH 8, 100 mmol/L NaCl, and 1 mmol/L EDTA.

### Bacterial Expression of the C-terminal End of G Protein

The pGEM-4-derived plasmids containing the C-terminal half of the G protein gene from the viral strains indicated earlier have been described [García et al., 1994]. These plasmids were used to amplify the terminal 3′ end of the G genes, corresponding to the last 84 amino acids of the gene in the case of strains encoding G proteins of 297 amino acids or the last 85 amino acids in the case of strains with G proteins of 298 amino acids, as previously described [Cane et al., 1996]. Oligonucleotides used as primers contained appropriate restriction sites. The PCR fragments were cloned initially into Bluescribe and then subcloned into the GST gene fusion vector pGEX-5X-3 (Pharmacia, Gaithersburg, MD), using *Bam*HI and *Sal*I sites [Cane et al., 1996]. Expression was induced by IPTG, and purification of the fusion proteins was carried out according to the manufacturer's protocols. The presence of G protein sequences in the fusion polypeptides was checked by immunoblot with specific MAbs.

### Immunoblot

Proteins were separated on 12% or 15% polyacrylamide gels under reducing conditions and electrotransferred to Immobilon paper (Millipore Corp., Bedford, MA). The blots were blocked with 0.2% I-BLOCK (Promega, Madison, WI) and 0.1% Tween 20 in PBS and incubated with sera. Antigen–antibody complexes were developed with biotinylated anti-human immunoglobulin and streptavidin–peroxidase using ECL chemiluminiscent reagent (Amersham, England) as substrate, following the manufacturer's instructions.

### Enzyme-linked Immunosorbent Assays

***Direct.*** Human serum samples were titrated by an ELISA using an extract of cells infected with the HRSV strain Mon/3/88 as antigen and 2% pig serum and 0.05% Tween 20 in PBS as blocking solution. Dilutions of sera were made in blocking solution. Bound antibodies were developed with biotin-labeled anti-human immunoglobulin, streptavidin–peroxidase, and OPD as substrate, following the manufacturer's instructions (Amersham).

***Competitive.*** MAbs were titrated by a direct ELISA, as described earlier. The antibody dilution that gave 80% of the maximum value was incubated with different dilutions of human sera and retested. Bound antibodies were developed with biotin-labeled anti-mouse immunoglobulin, streptavidin–peroxidase, and OPD as substrate. Titres of the competing antisera were calculated from the reciprocal of the serum dilution that gave 50% of the absorbance value obtained in their absence.

## RESULTS AND DISCUSSION

### Analysis of the Specificities of Anti-G Antibodies Present in Human Convalescent-phase Sera

Thirty-five convalescent-phase serum samples from young children and old people, with high anti-HRSV titres, were tested in a competitive ELISA with murine MAbs specific to the three types of epitopes identified in the G molecule (conserved, group specific, and strain specific). A schematic representation of the G protein with the location of MAb epitopes is shown in Fig. 1.

Representative examples of antibody competition obtained with 10 human serum samples are shown in

TABLE I. Competition of Antibodies Present in Human Sera for Antigen Binding With Anti-G Monoclonal Antibodies*

| Serum sample no. | Age of patients (years) | αHRSV titre[a] | Competitor monoclonal antibody[b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Conserved epitopes | | Group-specific epitopes | | Strain-specific epitopes | | | | |
| | | | 021/1G | 021/21G | 021/18G | 021/19G | 021/5G | 021/7G | 021/8G | 021/9G | 021/16G |
| 1 | 8 | 52,500 | 190 | 130 | 10 | 10 | – | – | – | – | – |
| 2 | 2 | >153,600 | 370 | 260 | 30 | 250 | – | – | – | – | – |
| 4 | 72 | 11,220 | 20 | 20 | 120 | 50 | – | – | – | – | – |
| 7 | 3 | 125,900 | 20 | 20 | – | – | – | – | – | – | – |
| 8 | 6 | 70,800 | 160 | 100 | – | 20 | – | – | – | – | – |
| 18 | 2 1/2 | 63,100 | 20 | 90 | 500 | 200 | 10 | – | – | – | – |
| 19 | 9 | >153,600 | 150 | 440 | 2,140 | 1,860 | 50 | – | 30 | – | – |
| 22 | 2 | >153,600 | 180 | 890 | 3,630 | 2,880 | 60 | – | – | – | – |
| 27 | 70 | >153,600 | 200 | 450 | 3,470 | 1,120 | – | – | – | – | – |
| 32 | 4 | 17,800 | – | – | – | – | – | – | – | – | – |

*The minus sign indicates no competition.
[a]HRSV, human respiratory syncytial virus. The enzyme-linked immunosorbent assay titre is the reciprocal of serum dilution giving 50% of the maximal value.
[b]Numbers refer to the titre of each serum sample in the competitive enzyme-linked immunosorbent assay, as indicated in Materials and Methods.

Table I. There was no correlation between serum titres in the competitive ELISA and titres in a direct ELISA designed to test anti-HRSV antibodies. For instance, serum sample no. 7 competed poorly with antibodies 021/1G and /21G and did not compete with any of the group-specific or strain-specific antibodies, despite having a high anti-HRSV titre (1:125,000). In addition, no correlation could be found between the serum titres obtained in the competition with different antibodies. For instance, serum samples nos. 1 and 8 competed efficiently with antibodies 021/1G and /21G but poorly with antibodies 021/18G and /19G.

A summary of the competition results of the 35 serum samples with anti-G Mabs corresponding to the three different types of epitopes is shown in Table II. Most sera showed significant antibody titres when tested for virus-binding inhibition of murine MAbs directed against conserved (021/1G and /21G) or group-specific (021/18G and /19G) epitopes. In contrast, only a limited number of serum samples competed with strain-specific antibodies. Thus, only four of 35 samples contained antibodies that inhibited binding of MAb 021/5G, mapped in the first hypervariable domain of the G protein (Fig. 1); one serum sample competed with MAb 021/8G, and none competed with MAbs 021/7G, /9G, or /16G.

The results presented in Tables I and II indicate that epitopes of the G protein, recognised by murine MAbs, are relevant to the human immune response to natural infections, but differences in specificities are found among distinct individuals. The lack of competition of most sera with strain-specific MAbs might be the result of amino acid sequence changes of those epitopes in the infecting viruses.

## Expression of the G-protein C-terminal Third in Bacteria and in Eukaryotic Cells

To test the reactivity of different serum samples with the hypervariable C-terminal segment of the G protein from different virus isolates, this segment either was expressed as a GST fusion protein in bacteria or was obtained as a proteolytic fragment of Gs purified from eukaryotic cells. The segment of the G-protein gene encoding the C-terminal 84-85 amino acids of Mon/3/88, Mad/2/88, Mad/3/89, Mad/5/92, Mad/8/92, and Long viruses, was cloned in the vector pGEX-5X-3. Authenticity of the plasmid constructs was confirmed by nucleotide sequencing. Following expression in *Escherichia coli* TG1 cells as soluble GST fusion proteins, 40–400 μg of purified protein was obtained after affinity chromatography using glutathione-Sepharose.

The soluble form of the G protein was purified by immunoaffinity chromatography from supernatants of Hep-2 cells infected with the previously mentioned viruses. Following incubation of Gs with V8 protease, two fragments (50 kd and 35 kd for the Long strain) were generated that reacted in immunoblot with a pool of anti-G MAbs (Fig. 2). Subtle differences in the electrophoretic mobility of the Gs fragments from different viruses are likely to reflect the amino acid and glycosylation changes between those virus isolates. Reactivity of the protease-resistant fragments from Long Gs with well-characterised MAbs indicated that the large fragment spanned epitope 63G (residue 204) to the end of the G polypeptide, whereas the small fragment represented the N-terminal half of the large fragment (Fig. 1) [García-Beato and Melero, unpublished observations]. Thus, the 50-kd fragments represent the glycosylated versions of the G protein segments that were expressed as GST fusion proteins.

## Reactivity of Human Sera With the Glycosylated or Nonglycosylated C-terminal End of the G Protein

Protease-digested Gs proteins, obtained from different virus isolates, and the corresponding nonglycosylated C-terminal segments, expressed as GST fusion proteins, were tested by Western blot with the 10 human convalescent-phase serum samples cited in Table

TABLE II. Summary of the Results of Competition of Sera with Anti-G Monoclonal Antibodies for Antigen Binding*

| Serum sample no. | Competitor monoclonal antibody: Conserved epitopes | Group-specific epitopes | Strain-specific epitopes |
|---|---|---|---|
| 3, **18, 19, 22** | + | + | + |
| **2,** 5[a], **8**[a], 9[a], 12[a], 13, 14, 15, 16, 17[a], 20, 21, 23, 24, 25[a], 26, **27,** 28[a], 29, 30[a], 33, 35 | + | + | – |
| **1, 7,** 31 | + | – | – |
| **4** | – | + | – |
| 6, 10, 11, **32,** 34 | – | – | – |

*The plus sign denotes competition and the minus sign lack of competition for antigen binding. Serum samples were grouped according to their competition with monoclonal antibodies representative of the different types of epitopes. Serum samples included in Table I are in boldface.
[a]These serum samples competed with only one of the group-specific monoclonal antibodies tested.

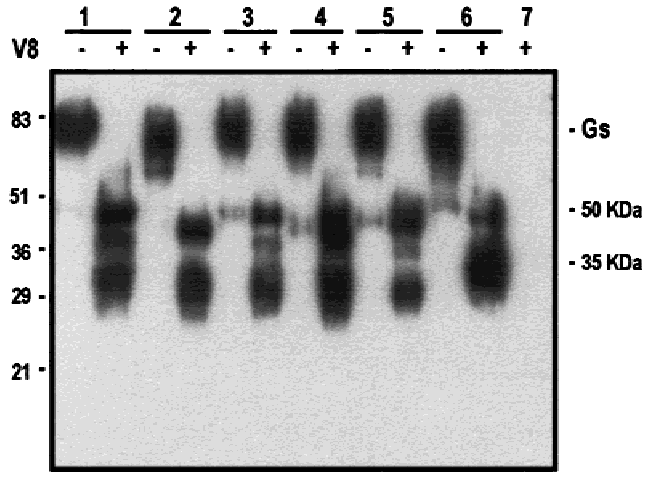


Fig. 2. Immunoblot of V8-digested Gs protein of different strains. The same amounts of Gs protein purified from culture medium of cells infected with the following viruses were applied to sodium dodecyl sulfate–polyacrylamide gel electrophoresis:1, Mon/3/88; 2, Mad/2/88; 3, Mad/3/89; 4, Mad/5/92; 5, Mad/8/92; 6, Long. Incubation with (+) or without (–) V8 protease is indicated. Lane 7 was loaded with V8 protease only. After electrotransfer to membranes, these were developed with a mixture of MAb 021/8G, 021/9G, 68G, and 63G. The location of Gs and relevant fragments is shown at right and molecular weight markers at left.

I. Illustrative examples are shown in Fig. 3, and a summary of results is shown in Fig. 4.

Whereas all serum samples reacted with the Gs protein of the strains tested, some samples failed to recognise any of the Gs protease fragments (e.g., sample no. 2, Fig. 3A) or reacted nonspecifically with the V8 protease used for Gs digestion (sample no. 1, Fig. 3A). In contrast, other serum samples, such as no.18, reacted with the 50-kd fragment of some strains but, in addition, recognised small fragments of the same or different strains that run faster than the 35-kd fragment (Fig. 3A). Serum sample no. 27 reacted differently with both the 50-kd and the 35-kd Gs protease fragments of certain strains. For instance, it recognised the 35-kd fragment but not the 50-kd fragment from the Long strain. Since the 35-kd fragment is included in the 50-kd fragment, the result obtained with sample no. 27 suggests that expression of certain epitopes present in the small fragment may be masked in the large fragment. We also have found that expression of certain epitopes located in the 35-kd fragment, and recognised by murine anti-G MAbs, are masked by cell-type-specific glycosylations of the 50-kd fragment [García-Beato and Melero, unpublished observations]. These results highlight the complexity of antibody specificities found in human convalescent-phase sera and the differences in serum reactivity with the Gs fragments derived from different viruses.

When the same serum samples were tested with the GST fusion proteins containing the nonglycosylated C-terminal third of the G protein, it was found that certain samples, such as no. 1, reacted with the protein derived from the six strains tested (Fig. 3B), even though they did not react with the glycosylated 50- kd fragment. Similarly, sample no. 2 reacted with the GST fusion proteins derived from certain strains, though it did not react with the 50-kd Gs protease fragment of those viruses. This finding indicates that epitopes of the G-protein C-terminal third, and recognised by certain sera, can be masked by sugars in the corresponding glycosylated G-protein fragment.

Figure 4 shows a summary of the reactivity of the 10 human serum samples of Table I with the Gs proteins, the Gs 50-kd fragments, and GST fusion proteins derived from the six HRSV strains tested. It should be stressed that the 50-kd fragment is equivalent to the segment expressed as GST fusion protein. All serum samples recognised the full-lengh Gs protein of the different strains, in agreement with the competition for virus binding with MAbs that recognised conserved and/or group-specific epitopes (Table I). Even serum sample no. 32, which did not compete with any of the MAbs of Table I, recognised the Gs protein of the six strains tested (Fig. 4). Thus, human sera may contain antibodies reacting with conserved epitopes that are not represented in the MAb panel.

Although all human serum samples reacted with the Gs protein of the six viruses, the majority reacted only with a limited subset of the 50-kd fragments derived from the different viral strains. This finding indicates that antigenic variation of HRSV isolates in this segment may be an important factor when evaluating the

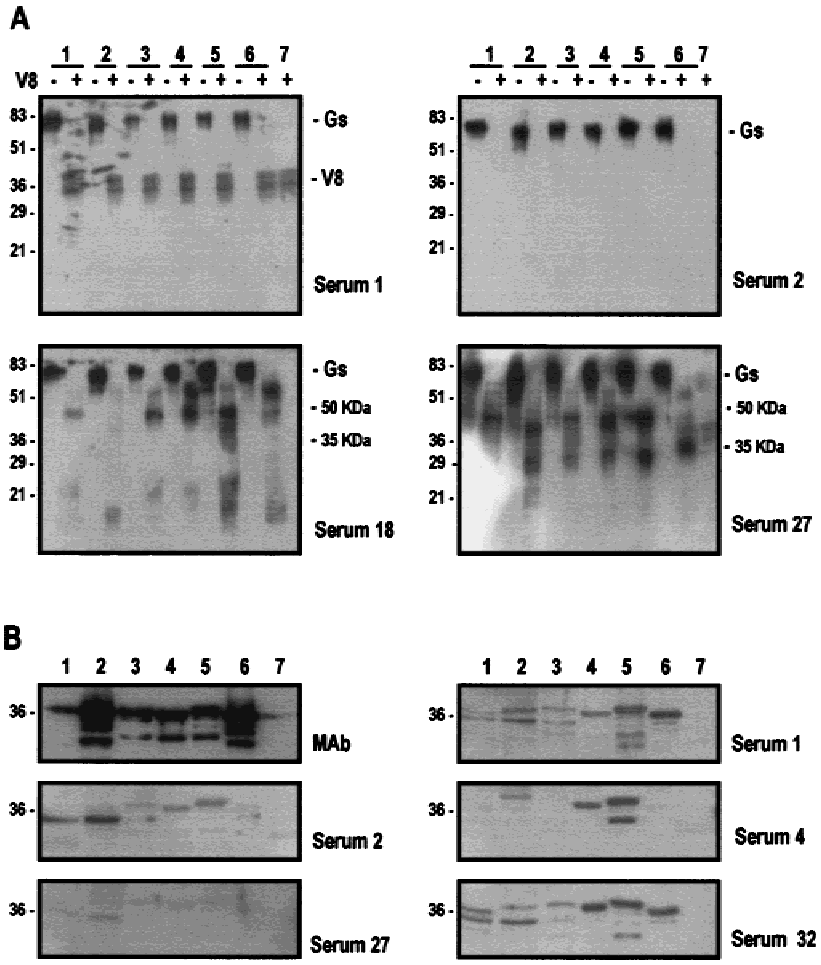


Fig. 3. Immunoblots of G protein derivatives from different HRSV strains with human sera. **A:** Gs proteins treated (+) or untreated (–) with V8 protease were processed as in Fig. 2. The fragments obtained were tested for reactivity with human sera. 1: Mon/3/88; 2: Mad/2/88; 3: Mad/3/89; 4: Mad/5/92; 5: Mad/8/92; 6: Long; 7: V8 protease. **B:** Fusion proteins of GST and the C-terminal third of the G protein of different HRSV strains (1–6 as in A; 7 GST only) were affinity purified and tested for reactivity with a mixture of MAbs (021/8G, 021/9G, 25G) or with human sera. The 36-kd molecular weight marker is shown in the left-hand margin.

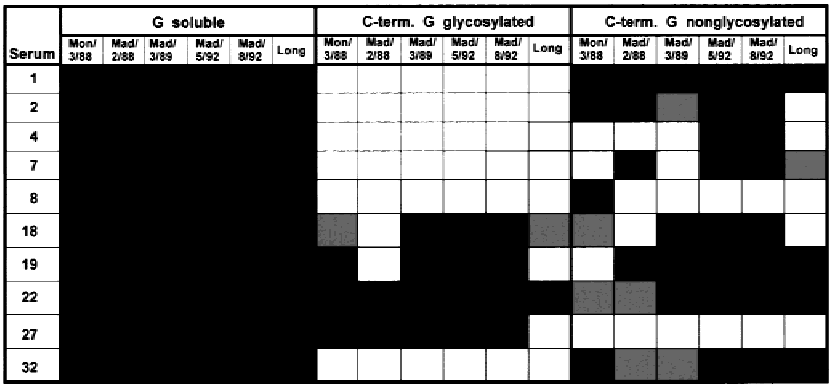


Fig. 4. Reactivity of human sera with different G-protein derivatives. G-protein derivatives corresponding to Gs protein, the 50-kd glycosylated Gs fragment, and nonglycosylated GST fusion proteins were tested as in Fig. 3 with the human sera indicated at left. ■, strong reactivity; ▨, weak reactivity; □, no reactivity.

repertoire of antibody specificities present in human sera. Strikingly, a high proportion of serum samples showed no reactivity with the 50-kd Gs fragment, despite reacting with the same segment expressed as GST fusion protein (e.g., serum samples nos. 1, 2, 4, 7, 8, and 32). In this regard, Wagner et al. [1986] have shown that the humoral immune response in humans against the HRSV G molecule is primarily IgG1 and IgG3, indicating that the protein moiety might be immunodominant. In contrast, sample no. 27, as mentioned earlier, reacted with the 50-kd fragment of five of six viruses but not with the corresponding GST fusion protein derived from them. Thus, the presence of carbohydrates in the C-terminal third of the G protein seems to influence the expression of certain epitopes in both directions, either masking the epitopes or contributing to antibody recognition.

In summary, the specificities of the human humoral immune response against the G protein of HRSV are highly heterogeneous, showing no clear correlation with age, clinical features, or titre in ELISA. The contribution of carbohydrates to the antigenic structure of

the G protein C-terminal third is of crucial importance, as is the genetic variability found among viruses in that segment of the attachment protein. Further studies should clarify the relevance of these findings for induction of a protective immune state against HRSV infection.

## ACKNOWLEDGMENTS

We thank Dr. B. García-Barreno for critical reading of the manuscript and Anna Simpson for technical help. Patients' serum samples were a kind gift of the Centro Nacional de Microbiologia, Majadahonda, Madrid. This work was funded by the following grants: European Union grant ERBIC18CT980374 (P.C. and J.A.M.) and PM 96-0025 from Ministerio de Educación y Cultura and 98/1086 from Fondo de Investigaciones Sanitarias (J.A.M.) and the Wellcome Trust (P.C.).

## REFERENCES

Anderson LJ, Hierholzer JC, Tsou C, Hendry RM, Fernie BF, Stone Y, McIntosh K. 1985. Antigenic characterization of respiratory syncytial virus strains with monoclonal antibodies. J Infect Dis 151: 626–633.

Cane PA. 1997. Analysis of linear epitopes recognised by the primary human antibody response to a variable region of the attachment (G) protein of respiratory syncytial virus. J Med Virol 51:297–304.

Cane PA, Matthews DA, Pringle CR. 1991. Identification of variable domains of the attachment (G) protein of subgroup A respiratory syncytial viruses. J Gen Virol 72:2091–2096.

Cane PA, Thomas HM, Simpson AF, Evans JE, Hart CA, Pringle CR. 1996. Analysis of the human serological immune response to a variable region of the attachment (G) protein of respiratory syncytial virus during primary infection. J Med Virol 48:253–261.

Collins PL, Mottet G. 1992. Oligomerization and post-translational processing of glycoprotein G of human respiratory syncytial virus: altered O-glycosilation in the presence of brefeldin A. J Gen Virol 73:849–863.

Collins PL, McIntosh K, Chanock RM. 1996. Respiratory syncytial virus. In: Fields BN, Knipe DM, Howley PM, editors. Fields Virology, 3rd ed. Philadelphia: Lippincott–Raven Publishers. p 1313–1351.

García O, Martín M, Dopazo J, Arbiza J, Frabasile S, Russi J, Hortal M, Perez-Breña P, Martínez I, García-Barreno B, Melero JA. 1994. Evolutionary pattern of human respiratory syncytial virus (subgroup A): cocirculating lineages and correlation of genetic and antigenic changes in the G glycoprotein. J Virol 68:5448–5459.

García-Barreno B, Jorcano JL, Aukenbauer T, López-Galíndez C, Melero JA. 1988. Participation of cytoskeletal intermediate filaments in the infectious cycle of human respiratory syncytial virus (RSV). Virus Res 9:307–322.

García-Barreno B, Palomo C, Peñas C, Delgado T, Perez-Breña P, Melero JA. 1989. Marked differences in the antigenic structure of human respiratory syncytial virus F and G glycoproteins. J Virol 63:925–932.

García-Beato R, Martínez I, Francí C, Real FX, García-Barreno B, Melero JA. 1996. Host cell effect upon glycosylation and antigenicity of human respiratory syncytial virus G glycoprotein. Virology 221:301–309.

Hendricks DA, McIntosh K, Patterson JL. 1988. Further characterization of the soluble form of the G glycoprotein of respiratory syncytial virus. J Virol 62:2228–2233.

Johnson PR, Spriggs RK, Olmsted RA, Collins PL. 1987. The G glycoprotein of human respiratory syncytial viruses of subgroups A and B: extensive sequence divergence between antigenically related proteins. Proc Natl Acad Sci U S A 84:5625–5629.

Martínez I, Dopazo J, Melero JA. 1997. Antigenic structure of the human respiratory syncytial virus G glycoprotein and relevance of hypermutation events for the generation of antigenic variants. J Gen Virol 78:2419–2429.

Mufson MA, Örvell C, Rafnar B, Norrby E. 1985. Two distinct subtypes of human respiratory syncytial virus. J Gen Virol 66:2111–2124.

Norrby E, Mufson MA, Alexander H, Houghten RA, Lerner RA. 1987. Site-directed serology with synthetic peptides representing the large glycoprotein G of respiratory syncytyal virus. Proc Natl Acad Sci U S A 84:6572–6576.

Palomo C, García-Barreno B, Peñas C, Melero JA. 1991. The G protein of human respiratory syncytial virus: significance of carbohydrate side-chains and the C-terminal end to its antigenicity. J Gen Virol 72:669–675.

Roberts SR, Lichtenstein D, Ball LA, Wertz GW. 1994. The membrane-associated and secreted forms of the respiratory syncytial virus attachment glycoprotein G are synthesized from alternative initiation codons. J Virol 68:4538–4546.

Sullender WM, Mufson MA, Anderson LJ, Wertz GW. 1991. Genetic diversity of the attachment protein of subgroup B respiratory syncytial virus. J Virol 65:5425–5434.

Wagner DK, Graham BS, Wright PF, Walsh EE, Kim HW, Reimer CB, Nelson DL, Chanock RM, Murphy BR. 1986. Serum immunoglobulin G antibody subclass responses to respiratory syncytial virus F and G glycoproteins after primary infection. J Clin Microbiol 24:304–306.

Wertz GW, Krieger M, Ball LA. 1989. Structure and cell surface maturation of the attachment glycoprotein of human respiratory syncytial virus in a cell line deficient in O-glycosilation. J Virol 63: 4767–4776.